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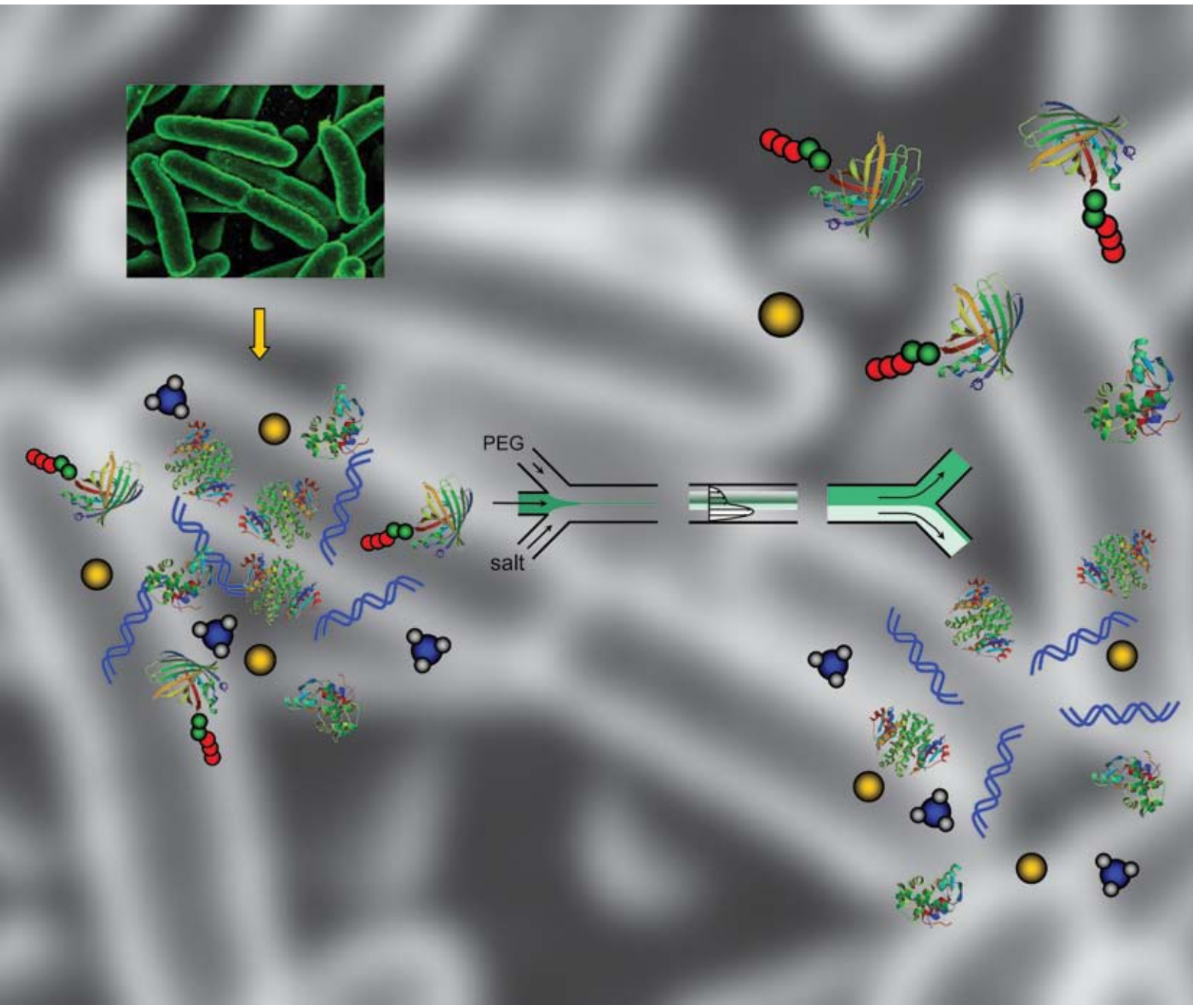
Volume 8 | Number 4 | April 2008 | Pages 501–632



ISSN 1473-0197

RSC Publishing

1473-0197(2008)8:4;1-#

Rapid, continuous purification of proteins in a microfluidic device using genetically-engineered partition tags†

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Received 25th October 2007, Accepted 24th January 2008
First published as an Advance Article on the web 25th February 2008
DOI: 10.1039/b716462a

High-throughput screening assays of native and recombinant proteins are increasingly crucial in life science research, including fields such as drug screening and enzyme engineering. These assays are typically highly parallel, and require minute amounts of purified protein per assay. To address this need, we have developed a rapid, automated microscale process for isolating specific proteins from sub-microlitre volumes of *E. Coli* cell lysate. Recombinant proteins are genetically tagged to drive partitioning into the PEG-rich phase of a flowing aqueous two-phase system, which removes ~85% of contaminating proteins, as well as unwanted nucleic acids and cell debris, on a simple microfluidic device. Inclusion of the genetic tag roughly triples recovery of the autofluorescent protein AcGFP1, and also significantly improves recovery of the enzyme glutathione S-transferase (GST), from nearly zero recovery for the wild-type enzyme, up to 40% with genetic tagging. The extraction process operates continuously, with only a single step from cell lysate to purified protein, and does not require expensive affinity reagents or troublesome chromatographic steps. The two-phase system is mild and does not disrupt protein function, as evidenced by recovery of active enzymes and functional fluorescent protein from our microfluidic process. The microfluidic aqueous two-phase extraction forms the core component of an integrated lab-on-a-chip device comprising cell culture, lysis, purification and analysis on a single device.

Introduction

Emerging research areas in biology and biotechnology, such as genomics, proteomics and structural biology increasingly require larger numbers of experiments performed in a smaller amount of time.[1,2] Moreover, these ever-increasing numbers of experiments often need to be performed using a limited amount of starting biological material. Examples include design of protein-based drugs and target screening,[3–5] engineering of enzymes for enhanced or novel activities,[6] and fundamental studies in proteomics or protein–protein interaction.[1,7] Microwell cell culture platforms enable production of microgram quantities of proteins using prokaryotic and eukaryotic hosts.[8–10] However, conventional bench-scale methods for purifying recombinant proteins often involve chromatography or centrifugation, which are labor-intensive and scale poorly for processing large numbers of small samples.[11,12] Poor sample recovery or unacceptable dilution often accompany attempts to purify microgram protein samples on equipment designed to process milligram quantities. Microfluidic techniques, on the other hand, have numerous advantages for processing small protein samples, including an inherent ability to process small volumes, precise control over fluid flow and surface properties, and the possibility for parallel operations. A single microfluidic device could integrate multiple steps, including a purification step as well as a microscale analysis, such as an activity measurement or crystallization screen. Microfluidic chips also offer scalability, with 8 or 12 channels on a single device at minimal added cost.

Microfluidic approaches for protein purification and analysis tend to focus on techniques such as chromatography, affinity capture and electrophoresis. Protein purification is a complex and difficult problem that is not generally amenable to a one-size-fits-all approach, and additional tools are currently needed. We present, using a combination of genetically-engineered partition tags and microfluidic processing, a microscale version of a PEG–salt aqueous two-phase extraction process. Microgram amounts of proteins are purified in minutes, with minimal loss of protein or activity. The method is applicable to any soluble protein that can be produced by genetic engineering in prokaryotic or eukaryotic hosts. The protein of interest is genetically modified to express a partition-tag, and the protein is expressed in microwells. Upon lysis, the cell lysate is introduced into a microfluidic device between the phases of a biocompatible two-phase flow. The protein of interest is extracted into one phase while DNA and other non-tagged proteins stay in the other phase and are discarded. The microfluidic scheme is rapid and continuous, with no need for expensive affinity reagents or packed columns.

Extraction in aqueous two-phase systems (ATPS) has long been used both on a laboratory bench scale and an industrial

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† Electronic supplementary information (ESI) available: Detailed procedures for cloning and protein expression; experimental data on analyte spreading during microfluidic extraction; video illustrating interface stability in a long microchannel. See DOI: 10.1039/b716462a

scale for separation and purification of proteins, including recombinant enzymes and protein-based drugs, from cell lysate.[13–15] PEG–salt two-phase systems are widely used in large-scale operations, providing good yields and high selectivity for desired proteins in the PEG-rich phase, while rejecting the majority of contaminants (cell wall debris, nucleic acids, many proteins, and unwanted enzymatic activities) into the salt-rich phase[15,16] At the bench scale, liquid–liquid extraction is typically a low-throughput batch technique, but it is well-suited for continuous operation on microfluidic chips, as low Re-number flows enable facile and reproducible generation of stable interfaces and two-phase flow.[17–19]

Most previous demonstrations of liquid–liquid extraction on chips have involved aqueous–organic phase systems, with partitioning of small molecules between the phases. Achieving stable, side-by-side two-phase flow with these systems often requires addition of surfactants to reduce interfacial tension,[20,21] or patterned surface treatments.[22–24] Solvent-permeable membranes have also been used to perform membrane-assisted extraction in microfluidic devices.[25,26] Regardless of the flow configuration, aqueous–organic systems tend to disrupt protein structure, and are generally not suitable for protein purification. ATPS are ideal for two-phase flow on microfluidic chips, as the low interfacial tension of these systems results in high capillary number even at low flow rates, making it straightforward to establish a stable interface without surfactants or special surface treatments. Previous applications of aqueous two-phase extraction on chips include simple protein fractionation,[27] fractionation of live and dead cells,[28] and electrophoretically-enhanced protein extraction.[29]

In conventional extraction, one relies on the inherent partition coefficient of the protein of interest, which makes it difficult to apply to proteins whose partition behavior is not known *a priori*. To circumvent this problem, we used genetic engineering to introduce short, hydrophobic tags to a protein of interest, which strongly biases partitioning into the PEG-rich phase. The partition tags are typically small (4–8 amino acids in length), and can be integrated seamlessly with other purification or expression tags, at either the *C*- or *N*- terminus. Previously reported partition tag sequences include $(WP)_4$, Y_3P_2, $(AWWP)_{1-3}$, and other variations rich in W or Y, typically with one or more P residues to ensure that the tag is located near the surface of the protein.[30–33]

Microfluidic extraction system

We demonstrate a microfluidic version of a single-stage PEG–salt aqueous two-phase extraction process that operates in a continuous (or semi-continuous) fashion, with genetic tagging of proteins to drive partitioning into the PEG phase. This operation is illustrated schematically in Fig. 1. A sample stream comprising a cell lysate with a desired (tagged) protein is hydrodynamically focused between two flowing streams containing PEG and potassium phosphate in sufficient concentration that the overall mixture forms two phases. The combination of laminar flow ($Re < 1$) and low interfacial tension (<0.1 mN m^{-1})[34] leads

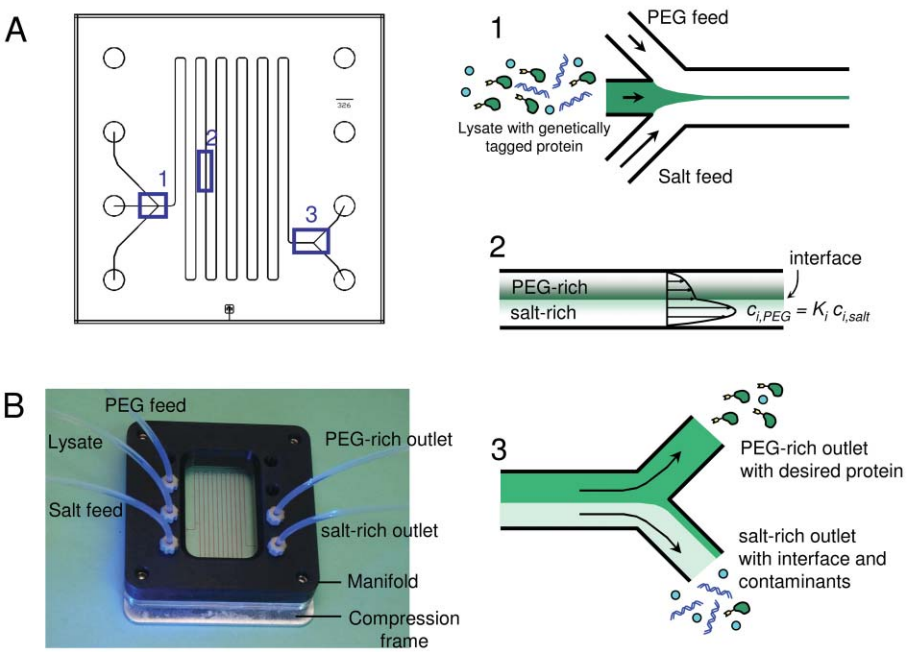

Fig. 1 (A) Schematic of laminar flow extraction. A typical microchannel design is shown with three inlets, a 326 mm serpentine channel, and two outlets on a 37 mm square glass chip. In region 1, a cell lysate stream, containing a genetically tagged protein of interest along with undesired contaminants, is hydrodynamically focused between inlet streams containing PEG and salt (potassium phosphate, pH 8). In region 2, farther downstream, laminar two-phase flow with a stable interface is observed. Components from the cell lysate stream partition between the two phases, with the genetically tagged protein (green) strongly partitioning toward the PEG-rich phase. An approximate velocity profile is illustrated, showing higher flow velocity in the less viscous salt-rich phase. At the end of the channel (region 3), the flow is split into two outlet streams. Undesired proteins and slowly-diffusing macromolecules are directed toward the salt-rich outlet along with the phase interface, while the tagged protein is concentrated in the PEG-rich outlet stream. (B) Photograph of the microfluidic chip mounted in a Delrin manifold, with inlet and outlet tubing connections.

to a stable interface, with side-by-side or stratified flow of the two phases, except at vanishingly small flow rates. This flow configuration is easily obtained, and the interface is stable for long times and over the entire length of very long microchannels, as illustrated in supplementary video 1 in the ESI.†

As the two-phase mixture flows down the length of the microchannel, components in the cell lysate, which are originally localized near the phase boundary, spread across the microchannel by diffusion, and partition between the two phases. A genetic tag on a specific, desired recombinant protein dramatically increases the partition coefficient for that protein only. At the typical flow rates used in our system (on the order of 5–20 mm s^{-1}), diffusion of proteins is fairly slow relative to convection (*i.e.* the Peclet number is large), and thus long, winding channels are used to provide sufficient residence time for diffusion away from the interface. Larger macromolecules such as genomic DNA and insoluble particles (*e.g.* cell wall debris) diffuse even more slowly, and thus remain near the interface for the entire length of the microchannel. The flow stream is split into two separate fluid outlets at the end of the microchannel. A "clean" PEG-rich phase containing the protein of interest is collected in one outlet, while the other outlet gives a "waste" stream containing the salt-rich phase, the phase interface (with any associated slowly diffusing species), and a small amount of the PEG-rich phase.

Because of the large viscosity difference between the PEG-rich and salt-rich phases (approximately 8-fold for the PEG-4000 we tested), the flow velocity in the salt-rich phase is significantly higher, resulting in a much larger volume collected at the salt-rich outlet. The volumetric flow rate of the PEG-rich phase is typically only a factor of 1–2 larger than the cell lysate, meaning that proteins collected in the PEG-rich phase are not significantly diluted. Meanwhile, species that partition fairly evenly between the phases are largely removed in the salt-rich phase, simply due to the large discrepancy in flow rates.

Results and discussion

Several simple experiments were performed with fluorescent tracer molecules to visualize performance of the microfluidic extraction system. Two examples are shown in Fig. 2. In Fig. 2A, a small-molecule tracer (FITC) is shown being hydrodynamically focused between PEG-rich and salt-rich precursor streams. The FITC diffuses rapidly, reaching concentration equilibrium by the end of the microchannel. Fig. 2B illustrates fractionation of fluorescently labeled BSA (which prefers the salt-rich phase), and β-galactosidase (which strongly prefers the PEG-rich phase). The fluorescence intensity profiles below the micrographs graphically illustrate the spreading of the proteins away from the interface as the flow progresses downstream. Additional data for spreading of tracer molecules in the flow system are presented in supplementary Fig. S2 in the ESI.†

We genetically tagged proteins with two previously reported partitioning tag sequences, $(WP)_4$[31] and Y_3P_2,[33] and found that either of these tags can greatly enhance recovery of the two proteins we tested. The $(WP)_4$ and Y_3P_2 tags were appended to proteins that could be easily assayed for function off-chip, namely AcGFP1 (a monomeric green fluorescent protein from *Aequorea coerulescens*) and the enzyme glutathione S-transferase (GST) from *Schistosoma japonicum*. Cell lysate containing the expressed proteins was fed continuously to the microfluidic extraction chip, and the PEG-rich and salt-rich outlet streams were assayed for fluorescence or GST activity. Appending either the $(WP)_4$ or Y_3P_2 tag dramatically increased the partition coefficient of both proteins toward the PEG-rich phase, and substantially enhanced recovery of the intact protein. Results of these experiments are shown in Table 1; the effect on AcGFP1 partitioning is also illustrated in Fig. 3. For AcGFP1,

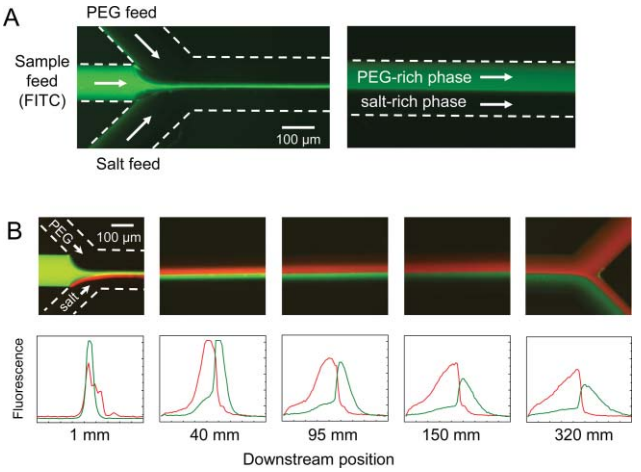

Fig. 2 Fluorescence micrographs illustrating aqueous two-phase extraction with fluorescently labeled analytes. (A) Fluorescence micrographs illustrating hydrodynamic focusing and partitioning of a FITC-containing sample stream. The left panel shows the channel intersection at the inlet of the chip, while the right panel shows the channel approximately 200 mm downstream. The FITC partitions strongly to the PEG-rich phase, highlighting the distinct boundary between the phases. (B) Fluorescence micrographs illustrating binary partitioning between BSA (Alexa Fluor 488 conjugate, green) and β-galactosidase (Alexa Fluor 555 conjugate, red), at a total flow rate of 7 μL min^{-1}. The plots below the micrographs are line profiles of fluorescence intensity across the width of the microchannel (total distance ~180 μm). Note the red "streak" near the microchannel inlet (left panel) is due to labeled β-galactosidase that had precipitated or adsorbed to the surface during previous operation.

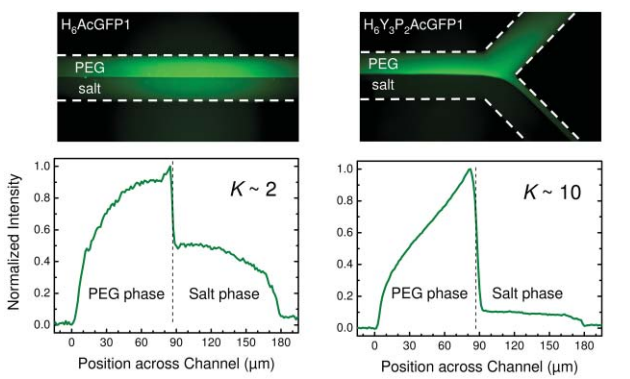

Fig. 3 Fluorescence micrographs illustrating partitioning of AcGFP1 with and without genetic modification with the Y_3P_2 partitioning tag. The micrographs were taken near the end of the channel, at ~320 mm from the inlet. The plots below the micrographs are normalized line profiles of fluorescence intensity, which can be used to calculate an apparent partition coefficient (K) for the AcGFP1.

Table 1 Performance of extraction chip for tagged and untagged biomolecules

Species	Format	K (off-chip)[a]	% Recovery in PEG phase[b]		Selectivity ratio (*versus* total protein)[c]
			Activity	Fluorescence	
AcGFP1 variants					
H_6-AcGFP1	Purified	1.5 ± 0.3 ($N = 7$)[d]		16 ± 3 ($N = 3$)	
H_6-$(WP)_4$-AcGFP1	Lysate	15 ± 3 ($N = 3$)		53 ± 12 ($N = 3$)	5
	Purified			92, 96 ($N = 2$)	
H_6-Y_3P_2-AcGFP1	Lysate	8.7 ± 0.1 ($N = 4$)		49 ± 18 ($N = 4$)	4
H_6-Y_3P_2-AcGFP1-P_2Y_3	Lysate	37 ± 20 ($N = 4$)		83, 86 ($N = 2$)	4
S. Japonicum GST variants					
GST, wild-type	Purified	<0.1[e]	1, 2 (N= 2)		
H_6-$(WP)_4$-GST	Lysate	>20[e]	34 ± 10 ($N = 5$)		5
	Purified		74, 78 ($N = 2$)		
H_6-Y_3P_2-GST	Lysate	>20[e]	45 ± 13 ($N = 4$)		3
H_6-Y_3P_2-GST-P_2Y_3	Lysate	>20[e]	40, 40 ($N = 2$)		
E. Coli β-galactosidase	Purified	110 ± 20 ($N = 3$)	50 ± 30 ($N = 3$)		
E. Coli total protein	Lysate	1.4 ± 0.3 ($N = 4$)		16 ± 5 ($N = 21$)	
E. Coli genomic DNA	Purified	0.05 ± 0.01 ($N = 3$)		0.2 ($N = 1$)	

[a] Partition coefficients and recoveries were measured using enzymatic activity assays for GST variants and β-galactosidase, fluorescence emission for AcGFP1, EZQ assay for total protein, and PicoGreen assay for DNA. Partition coefficients (K) were measured off-chip using a phase system made up of a 5 : 1 mixture of the salt and PEG feed streams mixed in a microtube, and centrifuged to separate the phases. Partition coefficient is defined as $K = C_{PEG}/C_{salt}$, where C refers to concentration as determined by activity or fluorescence measurements on at least 3 aliquots of each phase. [b] Recovery is the amount of activity, fluorescence, *etc.* in the PEG-rich outlet stream *versus* the total input during a period of collection. [c] Selectivity is the ratio of the recovery of the desired protein *versus* the recovery of the total protein content of the cell lysate, measured by EZQ assay for total protein on three 1 μL aliquots from each sample stream from each chip experiment. Standard deviation for three replicates of the EZQ total protein assay was commonly in the range of 5–15%. [d] For chip experiments, N is the number of separate on-chip extraction experiments performed. For each sample stream (inlet, PEG-rich outlet, and salt-rich outlet) from each chip extraction experiment, activity or fluorescence was typically measured on 2–3 aliquots from the collected volume. The relative standard deviations were typically ±10% for fluorescence measurements, and ±15% for activity measurements. [e] The GST assay used has limited dynamic range, making precise determination of very large (>20) or very small (<0.1) partition coefficients difficult. In these off-chip determinations of partition coefficient, GST activity was below the detection limit in one phase for 2–3 aliquots tested.

a single N-terminal $(WP)_4$ or Y_3P_2 modification gave a modest increase in recovery, with ~50 ± 15% recovery in 7 experiments for the tagged AcGFP1, *versus* 16 ± 3% for untagged AcGFP1. A Y_3P_2 modification at both termini of AcGFP1 gave still higher recovery (~85%) in two trials. For GST, all of the modifications tested provided similar recovery (~40 ± 10% recovery across 11 experiments) within the margin of error of the measurements. Wild-type GST was barely detectable in the PEG-rich outlet in two trials, and thus the tagged proteins represent a substantial improvement. With both AcGFP1 and GST, when the extraction experiment was performed with cell lysate, there tended to be some drop in total functional protein (*e.g.* total fluorescence or activity out was less than fluorescence or activity in the feed), indicating some tendency to destabilization or precipitation. This tendency was more pronounced for the $(WP)_4$ modification. Interestingly, when either $(WP)_4$-tagged protein was isolated from the cell lysate by affinity chromatography prior to the microfluidic extraction, recovery of activity or fluorescence was close to 100%, indicating that the loss of functional protein results from some interaction between the protein and other components of the cell lysate.

We also performed extraction experiments using (unlabeled) β-galactosidase, and measured β-galactosidase activity in the inlet stream and both outlet streams to demonstrate recovery of functional enzyme with strong inherent partitioning (*i.e.* without a genetic tag). Following a period of steady operation, material was aspirated from both outlets, and activity was measured off-chip using a fluorogenic assay. β-galactosidase activity was localized primarily in the PEG-rich outlet. The recovery depended on how closely the phases were split at the chip outlet (*i.e.* how much of the PEG-rich phase was directed to the salt-rich outlet along with the interface), with up to 80% of the β-galactosidase activity recovered in the PEG-rich outlet for an experiment with a very close split between the phases, but only 30–40% when the split was less precise.

The distribution of other cellular components was difficult to determine exactly due to the very small amounts of material involved. In experiments where *E. Coli* genomic DNA was spiked into the sample (to provide a sufficient amount of DNA for off-chip detection), the DNA was recovered quantitatively in the salt-rich outlet. In off-chip experiments with fluorescently labeled lipids, cell debris partitioned to the salt-rich phase. On-chip this material is difficult to track, but it appears to build up on channel walls (where it can be removed by rinsing between samples), or to stay near the interface and exit with the salt-rich stream. Total protein content of the *E. Coli* cell lines used for tagged protein expression partitions fairly evenly between the two phases (average $K \sim 1.4$ for 4 *E. Coli* cell lines expressing different recombinant tagged proteins). Despite the even partitioning, the much larger flow rate of the salt-rich stream leads to removal of 75–90% of total protein in this outlet. The selectivity for desired proteins (*e.g.* β-galactosidase or the tagged AcGFP1 or GST) relative to total protein content is thus typically in the range of 3–5. Higher selectivity with respect to total protein content has been reported with single-stage PEG–potassium phosphate systems (on a large scale) by operating at different positions on the phase diagram.[16] Because of the large viscosity difference between the PEG-rich and salt-rich phases, the simple chip design (with symmetric inlet and outlet channels) used here is somewhat restricted to operating in the

salt-rich corner of the PEG–salt phase diagram. Increasing the relative flow resistance of the salt-rich outlet channel would enable operation with a higher ratio of PEG-rich to salt-rich flows while still minimizing the loss of the PEG-rich phase to the salt-rich outlet.

We note that the margin of error on recoveries for the chip extraction experiments are fairly large. A variety of factors contribute to this uncertainty with the current setup: (1) exact and reproducible control of the split between the PEG-rich and salt-rich outlet streams is difficult with the current experimental design. (2) Dead volumes in chip wells along with significant swept volume in tubing result in long times required to establish steady-state operation. (3) The very small amounts of material used for on-chip experiments result in off-chip measurements of fluorescence or activity that are at the lower end of sensitivity for traditional bench-scale or microplate assays. The first and second of these factors are engineering challenges that can be addressed with improved design of the chip, manifold, and tubing connections, and with improved flow control. The third factor could be addressed with well-validated, chip-based assays scaled appropriately for very small quantities of analyte.

Clearly the microfluidic two-phase extraction does not provide a quantitative purification such as might be obtained by affinity chromatography. If the objective of the separation, however, is to clean up a sample sufficiently to lead into a more rigorous purification, or simply to remove certain unwanted or interfering proteins or activities prior to an on-chip assay, a single stage of aqueous two-phase extraction may be an excellent choice.

The microfluidic format can also be adapted to include a second stage of extraction, which can greatly improve the purity of the recovered protein. The chip design is simple, and does not require any on-chip fabrication or packing of particles. Two-phase systems can be tailored to recover specific proteins, or, as we have demonstrated, recombinant proteins in common expression vectors can be modified with simple fusion tags to greatly change their partitioning behavior. The throughput (sub-microlitre per minute) is well-suited to microscale assays currently being developed, and with further engineering, we expect microfluidic aqueous two-phase extraction to be a useful tool to incorporate into an integrated lab-on-a-chip device for high-throughput protein screening.

Materials and methods

Polyethylene glycol (MW 4000) and monobasic and dibasic potassium phosphate were purchased from Fluka. Stock solutions of PEG and potassium phosphate were prepared in DI water. Potassium phosphate solutions were prepared with a molar ratio of 3.56 : 1 dibasic : monobasic, resulting in a pH of approximately 8. For chip experiments, the PEG-rich inlet stream was 35 wt% PEG with 1.5 wt% potassium phosphate, and the salt-rich inlet stream was 16 wt% salt. PEG and salt flows were driven by pressurizing off-chip reservoirs with nitrogen using 0–15 psi scalable electronic pressure controllers (Parker Life Sciences), controlled by a PC with a LabVIEW interface. Protein or cell lysate samples were driven at 0.2 μL min^{-1} with a syringe pump (New Era) using a 100 μL syringe. Connections to the chip were made with 1/16″ OD × 0.01″ ID FEP tubing using in-house designed fittings. Typical upstream pressure for the PEG feed stream was 6 psig, with the salt feed pressure adjusted (typically 5.7–5.9 psig) to control the position of the interface. During experiments in which fluids were collected from open chip outlets, material was periodically aspirated to prevent buildup of a pressure differential between the outlets. Typical collection rates at ~6 psi pressure head were 0.3–0.4 μL min^{-1} of the PEG-rich outlet, and 2.5–3.5 μL min^{-1} of the salt-rich outlet.

Chip patterns were designed using AutoCAD LT, and a chrome mask was produced by Photo Sciences (Torrance, CA, USA), with a main channel line width of 80 μm. Chip fabrication (including photolithography, wet etch, bonding, and dicing) was performed by Caliper Life Sciences (Mountain View, CA, USA) on sodalime glass, with an isotropic etch depth of 50 μm, and 2.5-mm OD circular wells. Chips were mounted in a custom-manufactured Delrin manifold with threaded ports for tubing connections, with rubber O-rings and an aluminum compression frame to provide fluidic seals around the wells.

Cloning and protein expression of tagged proteins were performed using standard techniques as described in the ESI.† Purified β-galactosidase and *E. Coli* genomic DNA were obtained from Sigma, purified H_6-AcGFP1 was from Clontech, and purified recombinant *S. japonicum* GST was from Genscript. β-Galactosidase activity was measured off-chip using the FluoReporter lacZ/galactosidase kit from Invitrogen and a fluorescence microplate reader (Molecular Dynamics). GST activity was measured off-chip using the GST-Tag assay kit from Novagen, and a UV-VIS microplate reader (Molecular Dynamics). AcGFP1 fluorescence was quantified off-chip using a fluorescence microplate reader or fluorescence spectrophotometer (Perkin Elmer). Genomic DNA content in outlet streams was measured using PicoGreen reagent (Invitrogen) and a fluorescence microplate reader or spectrophotometer. Total protein content was assayed using the EZQ protein quantitation kit (Invitrogen) with a UV transilluminator and CCD camera for blot quantitation. The various protein and DNA assays and AcGFP1 fluorescence measurements were tested for the impact of varying PEG and potassium phosphate concentrations, and found to be insensitive at the dilutions used.

Chip extraction experiments were performed at least in duplicate. Assays were performed on 2–3 aliquots from each sample stream from each chip extraction experiment. Activity assays were performed simultaneously with a calibration of three or more concentrations of known standards. Three aliquots of each stream were tested if possible, but in some cases the small sample volume dictated splitting the entire sample into just two aliquots in order to obtain measurable signals in a standard 200 μL microplate assay or 2 mL fluorimeter cuvette. The margin of error for the different types of measurements based on replicate analyses of numerous samples and standards are estimated as follows: ±10% for fluorescence of AcGFP1, ±15% for total protein by EZQ assay, ±15% for GST and β-galactosidase activity assays, and ±5% for DNA assay by PicoGreen, when used within the appropriate range.

Conclusions

Aqueous two-phase microfluidic extraction is a flexible approach for purification of proteins from complex biological samples, and

is easy to implement in simple microchannels. By genetically tagging recombinant proteins with short partitioning tags, we greatly increase the selectivity for specific proteins. The PEG–salt system preserves protein structure sufficiently to maintain the intrinsic fluorescence of AcGFP1 and enzymatic activity for GST and β-galactosidase. The single-stage extraction format offers a modest increase in purity, and addition of further extraction stages on the same device is expected to improve performance further. Microfluidic extraction is envisioned as a key component in an automated high-throughput platform for protein analysis, comprising microscale cell culture, cell lysis, protein purification, and on-chip analysis. Researchers can select from a library of previously developed partitioning tags, including the Y_3P_2 and $(WP)_4$ modifications, as well as others reported in the literature. The extraction process was demonstrated here in reusable glass chips, but is easily amenable to inexpensive, mass-produced devices in material such as PMMA. The microfluidic extraction thus provides a means of micro-scale protein purification using a simple, disposable device, without requiring expensive affinity reagents or chromatographic supports.

Acknowledgements

We thank Ron Renzi at Sandia National Laboratories and Harvey Blanch at the University of California, Berkeley. Sandia is a multiprogram laboratory operated by Sandia Corp., a Lockheed Martin Co., for the U.S. Department of Energy under Contract DE-AC04–94AL85000. Funding was provided by the DOE Office of Science and the Sandia LDRD program.

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